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CLINICAL STUDY

Selected biomarkers of age-related diseases in older subjects with different nutrition

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Abstract: *Background:* The nutritionists introduce on the base of epidemiological and clinical studies that appropriately planned vegetarian diets are healthful, and may provide health benefits in the prevention and treatment of certain diseases. Aging belongs to the main risks of cardiovascular disease.
Methods: Markers of age-related diseases (cardiovascular, metabolic syndrome, diabetes) were assessed in two nutritional groups of older apparently healthy non-obese non-smoking women aged 60–70 years, 45 vegetarians (lacto-ovo-vegetarians and semi-vegetarians) and 38 non-vegetarians (control group on a traditional mixed diet, general population).
Results: Vegetarian values of total cholesterol, LDL-cholesterol, triacylglycerols, C-reactive protein, glucose, insulin and insulin resistance are significantly reduced. Non-vegetarian average values of total cholesterol, LDL-cholesterol and C-reactive protein are risk. Vegetarians have a better antioxidative status (significantly increased vitamin C, lipid-standardized vitamine E and β-carotene plasma concentrations).
Conclusion: Favourable values of cardiovascular risk markers in older vegetarian women document a beneficial effect of vegetarian nutrition in prevention of this disease as well as the vegetarian diet can be an additional factor in therapy. Vegetarians suffer from mild hyperhomocysteinemia; it is due to the lower vitamin B12 concentration. Vitamin B12 supplements are inevitable for the hyperhomocysteinemia prevention *(Tab. 2, Ref. 26)*. Full Text in free PDF *www.bmj.sk.*
Key words: cholesterol, C-reactive protein, insulin resistance, antioxidative vitamins, vegetarian nutrition.

The American dietetic association introduced that appropriately planned vegetarian diets are healthful, nutritionally adequate, and may provide health benefits in the prevention and treatment of certain diseases (1). A vegetarian diet is associated with many health benefits because of its higher content of fiber, folic acid, vitamins C and E, potassium, magnesium, and many phytochemicals and a fat content that is more unsaturated (2). On the other hand, vegetarian diets are described as deficient in several nutrients. Numerous studies have demonstrated that the observed deficiencies are usually due to poor meal planning (3). Vitamins B12, D and n-3 fatty acids are not contained in plant food. Contents of methionine, iodine and calcium are significantly reduced in comparison to animal sources. Iron, zinc and calcium may be of concern because of the limited bioavailability of these minerals (1, 4). From view of prevention of health risks of vegan diet or incorrectly planned vegetarian diet is the consumption of fortified food or nutritional and pharmacological supplements to compensation of absent or low-present nutrients in key vegetarian food inevitable (1, 5, 6).

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Cardiovascular diseases are very frequent diagnoses in subjects aged 65 years or older (7). Aging belongs to main risk factors of cardiovascular disease. More than 80 % of cardiovascular mortality and cardiovascular disease appears in persons aged 65 years and older. Gradual prolongation of life (8) requires an adequate health care in older or old age including the health life style, appropriate physical activity but also correct nutrition.

The main goal of this study was to assess the selected cardiovascular risk parameters in two groups of older subjects of different nutritional regimen.

Subjects and methods

Randomly selected 83 apparently healthy non-obese (BMI<30) non-smoking women aged 60-70 years (volunteers of epidemiological research of ageing processes) were divided into two groups. The group of long term vegetarians (n=45) consisted of 20 lacto-ovo-vegetarians, who consumed plant food, dairy products and eggs and 25 semi-vegetarians with addition of white meat and fish consumption ≤1-time weekly. Non-vegetarian (control) group consisted of 38 persons of general population on traditional mixed diet. The group characteristic is introduced in Table 1. The probands have an approximately similar physical activity (no sports).

Blood was sampled after an overnight fasting by a standard procedure. Serum concentrations of total cholesterol, HDL-cho-

Tab. 1. Group characteristics, cardiovascular risk markers and plasma antioxidative vitamins.

	Non-vegetarians	Vegetarians
n	38	45
age range (y)	60-70	60-70
average age (y)	63.4±0.5	65.2±0.5
BMI ($kg.m^{-1}$)	26.7±0.4	24.6±0.5^
BMI range ($kg.m^{-1}$)	21.7-29.8	18.7-28.7
duration of vegetar. (y)	–	10.3±0.8
blood pressure (mm Hg)		
systolic	162.8±3.6	147.7±2.8^
diastolic	80.1±1.7	77.8±1.4
total cholesterol (mmol/l)	5.82±0.24	5.20±0.09^
triacylglycerols (mmol/l)	1.54±0.13	1.29±0.07°
HDL-cholesterol (mmol/l)	1.53±0.06	1.54±0.06
LDL-cholesterol (mmol/l)	3.62±0.21	3.12±0.10°
atherogenic index	3.88±0.17	3.49±0.13
glucose (mmol/l)	5.76±0.18	5.50±0.10°
insulin (mU/l)	8.18±0.63	6.57±0.38°
insulin resistance IR(HOMA)	2.18±0.22	1.58±0.10°
C-reactive protein (mg/l)	2.47±0.41	1.09±0.14^
homocysteine (μmol/l)	13.7±0.8	17.5±0.8^
vitamin B12 (pmol/l)	328±22	211±16*
folic acid (nmol/l)	24.2±1.4	23.4±1.4
vitamin B6 (μg/l)	5.40±0.80	7.86±0.69°
conjugated dienes of fatty acids (μmol/l)	1.60±0.11	1.12±0.08*
vitamin C (μmol/l)	45.1±3.5	58.2±2.5^
vitamin E (μmol/l)	37.4±1.6	36.6±1.5
vitamin E/total cholesterol+ triacylglycerols (μmol/mmol)	5.09±0.15	5.63±0.19°
β-carotene (μmol/l)	0.237±0.025	0.559±0.057*

The results are expressed as mean±SEM
BMI - body mass index
° $p<0.05$, ^ $p<0.01$, * $p<0.001$

lesterol, triacylglycerols and glucose were measured using standard laboratory methods. Values of LDL-cholesterol were calculated according to the Friedewald formula. The atherogenic index = total cholesterol/HDL-cholesterol. Serum concentrations of insulin were detected by electro-chemiluminiscence immunoassay (Roche Elecsys Insulin Test). Insulin resistance values IR/HOMA/ (HOMA – homeostasis model assessment) were calculated from fasting concentrations of insulin and glucose: IR / HOMA/ = insulin x glucose/22.5. Serum C-reactive protein concentrations were detected by immunoturbidimetric method using a high sensitivity test (Randox, UK). Plasma concentrations of total homocysteine were measured by HPLC (9). EDTA was used as an anticoagulant. Serum vitamin B12 and folic acid concentrations were determined using Elecsys immunoassay (Roche tests). Serum vitamin B6 values were detected by HPLC method (Chromsystems test). Plasma concentrations of vitamins C and E and β-carotene were measured by HPLC (10, 11). Conjugated dienes of fatty acids in plasma were assessed spectrophotometrically (12). The intake of vitamins, mineral and trace elements was allowed only in natural form (no supplementation). The Student t-test was used for final evaluation.

The calculation of daily intake of nutrients was based on the data from dietary questionnaires. The questionnaire contained 146 food items. The frequency of consumption was measured using four categories: almost never, times per day, per week or per month depending on food item. Trained workers checked the completeness of questionnaires. The conversion to nutrients was done by using self-developed software Nutrition based on the Slovak food composition database compiled by the Food Research Institute (13).

Results and discussion

Consumption of saturated fat (animal sources) has been found to be associated with hypercholesterolemia, while polyunsaturated fats (plant sources) were reported to have a cholesterol lowering effect. Consumption of food high in dietary fibre is associated with a lower risk of cardiovascular disease because of the ability of soluble and insoluble fibres to reduce plasma total and LDL cholesterol. In addition to unsaturated fat and fibre, there are components of plant food that are known to reduce cardiovascular risk (saponins in legumes, plant proteins, antioxidant nutrients, selenium, polyphenols and flavonoids (14, 15, 16, 17)). Previously, the dietary approach to reducing cardiovascular risk was aimed at decreasing total and saturated fat intake from meat consumption. Actually, this alone may not be sufficient. The inclusion of a variety of plant foods is necessary to favourable modify lipid and lipoprotein profile (18).

Cardiovascular risk can be decreased by plant protein consumption. For a longer time, experimental studies described, that animal proteins with higher content of essential amino acids in comparison to plant proteins induce an elevation of plasma total and LDL cholesterol concentrations that can be prevented by a plant protein consumption (14). The higher intake of methion-

Tab. 2. Daily intake of selected nutrients and food commodities

	Non-vegetarians	Vegetarians
total fats (g)	82.9±4.7	76.8±3.1
cholesterol (mg)	266±18	121±10 *
saturated fatty acids (g)	32.2±1.0	21.1±0.8 *
monounsaturated fatty acids (g)	27.7±1.2	27.4±1.1
polyunsaturated fatty acids (g)	18.6±0.9	24.8±0.8 *
linoleic acid (g)	16.5±0.8	22.2±0.7 *
α-linolenic acid (g)	1.51±0.11	1.96±0.13 ^
fibre (g)	26.0±1.3	35.0±1.8 *
total proteins (g)	87.0±4.7	78.1±3.4
plant proteins (g)	40.2±2.3	50.3±2.7 ^
folic acid (μg)	366±16	397±19
vitamin B6 (mg)	1.97±0.09	2.30±0.11°
vitamin B12 (μg)	3.51±0.35	1.96±0.33 ^
vitamin C (mg)	97±4	149±11 *
vitamin E (mg)	11.3±0.8	12.4±0.7
β-carotene (mg)	4.07±0.23	6.41±0.43 *
whole grain products (g)	62.4±11.7	115.6±10.9 ^
pulses (g)	17.8±2.8	26.9±3.2 °
vegetables (g)	190±15	293±23 *
fruit (g)	251±19	383±25 *

The results are expressed as mean±SEM
° $p<0.05$, ^ $p<0.01$, * $p<0.001$

ine and lysine from animal proteins has an unfavourable effect on phospholipid metabolism (15).

Vegetarians in the present study consume a significantly reduced amount of saturated fatty acids and cholesterol (Tab. 2) and on the other hand, they have a higher daily intake of polyunsaturated fatty acids, linoleic acid and α-linolenic acid. Table 2 introduces also that vegetarians have significantly higher intake of fibre, plant proteins, whole grain products, legumes, fruit and vegetables, vitamin C and β-carotene. Favourable values of cardiovascular risk markers as a consequence of vegetarian nutrition are introduced in Table 1. Concentrations of total cholesterol, LDL-cholesterol and triacylglycerols are in vegetarians vs. non-vegetarians significantly reduced. Vegetarian average values of all lipid parameters are in reference range, in non-vegetarian group the risk average value of total cholesterol and LDL-cholesterol was found. Vegetarian vs. non-vegetarian women have a significantly lower systolic blood pressure and BMI. Vegetarian plasma concentrations of antioxidative vitamins C and β-carotene are significantly higher in comparison to non-vegetarians and they are in range of effective reduction of free radical disease (Tab. 1) (19). Plasma lipid-standardized values of α-tocopherol are also significantly higher in vegetarians. Alternative nutrition women have significantly reduced values of lipid peroxidation (conjugated dienes of fatty acids) (Tab. 1) though they consume higher amounts of polyunsaturated fatty acids with greater oxidation ability. This fact is a consequence of the better antioxidative status of vegetarian nutrition (20).

High sensitivity C-reactive protein (hsCRP), a marker of inflammation, confers additional prognostic value at all concentrations of cholesterol, Framingham coronary risk score, severity of the metabolic syndrome, blood pressure and in those with and without subclinical atherosclerosis (21). Vegetarian vs. non-vegetarian CRP values are significantly reduced (Tab. 1). Average value in vegetarians is on lower borderline of moderate risk (1–3 mg/l), while in non-vegetarians this value is nearly upper borderline of moderate risk. Low values of CRP in vegetarians are a consequence of regular and sufficient consumption of fruit and vegetables, which are important sources of dietary salicylates as well as other anti-inflammatory compounds.

Subjects consuming predominantly plant food may be at lower risk of type 2 diabetes occurrence than persons on traditional mixed diet (22). Complex carbohydrates with low glycemic index are slowly absorbed and thus they have a beneficial effect on glucose control, hyperinsulinemia, insulin resistance and blood lipids (23). Hyperinsulinemia and insulin resistance are critical components of the metabolic syndrome and are the early manifestations of type 2 diabetes. Vegetarian vs. non-vegetarian IR/HOMA/ value is significantly reduced (Tab. 1). Daily intake of crucial food with preventive metabolic syndrome or diabetes effect (whole grain products, legumes, fruit and vegetables) is significantly higher in vegetarians (Tab. 2).

Plant proteins have the potential to favourable influence glucagon and insulin activity (24). Plant proteins are higher in non-essential amino acids in comparison to animal protein sources (15). Essential amino acids are relatively more effective for releasing insulin, whereas non-essential amino acids are effective in glucagon secretion. The effect of a chronic increase in glucagon activity by regular and sufficient intake of plant proteins means a reduction of lipogenesis, decreasing fat storage, a reduction in cholesterol synthesis and in circulating LDL cholesterol, a reduction in triacylglycerol synthesis.

The favourable values of lipid and non-lipid cardiovascular risk markers in older vegetarian women document a beneficial effect of vegetarian nutrition in prevention of this disease as well as vegetarian nutrition can be an additional factor in therapy. The exception from markers is atherogenic homocysteine. Vegetarians suffer from mild hyperhomocysteinemia as a consequence of lower vitamin B12 intake (Tab. 2) and lower serum concentrations (Tab. 1). Vegetarian average HCy value is over risk limit (>15 μmol/l) and average vitamin B12 concentration is deficient (<220 pmol/l). In non-vegetarian women, intake of vitamin B12 is almost two-fold higher in comparison to vegetarians (a greater intake of animal food), serum concentration is over 220 pmol/l and HCy value is below 15 μmol/l. Third nutritional determinant of HCy metabolism vitamin B6 is significantly higher in vegetarians, but this vitamin is non-dominant in HCy degradation in normal weight subjects (25). During the last decade, several observational studies about HCy as a predictor for atherosclerosis risk showed that the overall risk for vascular disease is small (26). In prospective longitudinal studies, a weak association between HCy and atherothrombotic vascular disease was reported, compared to retrospective case-control and cross-sectional studies with stronger association. In spite of these findings hyperhomocysteinemia and vitamin B12 deficit weaken the protective effect of vegetarian diet. The correct nutritional regimen, monitoring of plasma vitamin B12 and using vitamin B12-fortified food or vitamin B12 supplements are inevitable for the prevention of hyperhomocysteinemia in vegetarians (5, 6).

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